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Parthenogenetic Mouse Embryonic Stem (mES) Cells Have Similar Characteristics to *In Vitro* Fertilization mES Cells

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This study was to compare the characteristics of parthenogenetic mES (P-mES) cells and *in vitro* fertilization mES cells. Mouse oocytes were recovered from superovulated 4wks hybrid F1 (C57BL/6xCBA/N) female mice. The oocytes were treated with 7% ethanol for 5 min and 5 μg/ml cytochalasin-B for 4 h. For IVF, the oocytes were inseminated with epididymal sperm of hybrid F1 male mice ($1x10^6$/ml). IVF and parthenogenetic embryos were cultured in M16 medium for 4 days. Cell number count in blastocysts was carried out differential labelling using propidium iodide (red) and bisbenzimide (blue). To establish mES cells, blastocysts in IVF and parthenogenetic groups were treated immunosurgery and recovered ICMs were cultured in LIF added DMEM culture medium. To identify mES cells, the surface markers alkaline phosphatase, SSEA-1, 3, 4 and Oct4 staining in replated ICM colonies were examined. Also, the number of chromosome was checked in P-mES and mES. Identification of neural cell and beating cadiomyocytes differentiation was carried out by immunocytochemistry using MAP-2 (Sigma), GFAP (DAKO) and Sarcomeric α-actinin. *In vitro* development rates were blastocysts derived from parthenogenetic group (14.5%) significantly lower than IVF group (68.0%) ($P<0.05$). And, cell numbers of ICM of parthenogenetic blastocysts (12.1) were lower than those of IVF blastocysts (23.0). Three ICM colony (P-mES01, 02 and 03) recovered from parthenogenetic 9 blastocysts and 1 ICM colony (mES01) recovered from IVF 26 blastocysts were subcultured and continuously replated during 20 passage and 15 passage culture duration without differentiation. Using surface markers staining, alkaline phosphatase, SSEA-1, 3, 4 and Oct4 in P-mES and mES colony were examined. Sub-cultured each group colonies were strong positively stained by alkaline phosphatase and SSEA-1 staining. And, negatively stained by SSEA-3, 4 staining. The number of chromosome was normal in ES colony from parthenogenetic group and IVF group. When P-mES02 colony was differentiated into neural cell and beating cardiomyocytes *in vitro*, it expressed MAP-2 (Sigma), GFAP (DAKO) in neural cells and Sarcomeric α-actinin in beating cardiomyocytes. This study suggested that P-mES cell can be successfully established and that those cell lines have similar characteristics to IVF mES cells. Also, neural cells and cardiomyocytes derived from the P-mES02 cells were *in vitro* differentiation.

Key words) ***Parthenogenetic, Mouse embryonic stem cell, Blastocysts***